MCDB 4426 Exam 2 3/23/06

Be concise. Excess words make graders grumpy. Name _____KEY_____

1. In the *lin-3* paper, Figure 2 schematizes the *lin-3* locus and includes many of the DNA constructs used in the experiments. Which DNA construct(s) is/are **most** useful in pinpointing the location of the gene, and why?

8pts
pRH19 is the most useful construct, it is the smallest construct that rescues the lin-3 mutant and causes the Muv phenotype

note- negative results (e.g. pRH23) are considerably weaker (less informative) than this positive result

for example, there are many reasons that a transgene might not rescue (e.g. damaged, silenced)

2. In the *lin-3* paper, the authors conclude that *lin-3* encodes a signaling molecule important in inducing vulval fate of the vulval precursor cells.
a) List 3 different pieces of evidence described in the paper that support this conclusion.

9pts

1) encodes a protein similar to EGFR ligands having a putative signal seq
2) promoter is expressed in Anchor Cell (lacZ transgene) not VPCs
3) transgenes induce Muv phenotype (VPCs become vulva rather than hypodermis)
4) ablation of gonad precursors in lin3::lacZ line prevents Muv
5) reducing lin3 activity causes Vul phenotype-lack of vulval fate

b) Which piece of evidence presented in the paper supporting the conclusion that *lin-3* is an inducing signal is the **strongest** and why?

6pts
Expression of the transgene in the AC induces a Muv phenotype (VPCs become vulval rather than hypodermal), and ablation of the AC in transgenic worms largely prevents this

3. In the Hafen et al. *sevenless* paper, **figure 4** is a schematic for the region the scientists focused on as containing the *sevenless* gene. Describe the relevance of each of the following to pinpointing the location of the *sevenless* gene:

1- $Df(1)^{v64f29}$

5 pts

a functional part of the gene must be proximal of the breakpoint of this Df because it complements sev

2- $Df(1)^{rasv17Cc8}$

5pts

An essential part of the sev gene must be contained within this Df because it fails to complement sev

3- HsPneo sev-15

5pts

because the genomic fragment in this transgene rescues sev, the gene must be contained within it

4. In the Simon et al paper…

a) There are a number of ommatidia that are "exceptions" indicated with arrows in Figure 3. Why does it make sense that these **exceptions** are present?

7pts

The system is poised at the edge of the amount of Sevenless signal necessary for specification of R7 in most ommatidia. Occasional cells would be expected to express more or less of pathway components, sev, etc., causing them to either become, or fail to become R7. Thus, stochastic fluctuations lead to exceptions.

b) In figure 5, photoreceptors other than R7 are affected. Why does this make sense?

7pts

The genes mutated to become Enhancers of the sevts must function in pathways that operate in multiple cell types- other than the sev pathway (recall that mutation of sev only leads to absence of R7). This makes sense because one might expect there to be components of tyrosine kinase signaling cascades shared by many different Receptor Tyrosine Kinases.

Many students re-stated the conclusion from the experiment. The question asks why the result makes sense.

c) In figure 7, several of the Enhancer mutations suppress *Ellipse*. Why does this make sense?

7pts

Like Sevenless, Ellipse encodes an RTK, but in this case Ellipse is mutated such that it is more active than normal. Thus, the sev Enhancers might be expected to function as Ellipse suppressors- if they function to reduce the amount of signal in a tyrosine kinase cascade by loss-of-function mutation of components used in multiple RTK cascades.

d) Describe 3 different types of evidence presented in this paper indicating that the gene encoding the guanine nucleotide exchange factor *Sos* corresponds to *E(sev)2A* (e.g. see figures 9 and 10). More credit will be given for answers using the 3 different strongest types of evidence.
9pts
1- the BglII-Xba fragment rescues the E(sev)2A lethal phenotype
2- there are mutations in the sos open reading frame in several different E(sev)2A alleles
3- the only transcript (cDNA) found in eye discs that was fully contained in the rescue fragment encodes the predicted sos protein
4- E(sev)2A mapped near black by meiotic recombination

e) In this paper, the authors identified components of the *sevenless* pathway that normally act to increase the amount of signal. How might they conduct a similar genetic screen, using the same temperature-sensitive *sev* transgene, but instead identify components that normally act to decrease the amount of signal?

8 pts
poise the inclubator so it is just above the restrictive temp (~24.3 degrees) so just barely sevenless

screen for suppressors that lead to the presence of R7

5. In the Christopherson et al. paper…
a) What result would be expected in Figure 1E if ACM and TSP1 induced puncta formation through different pathways?

6pts
ACM + TSP1 would lead to an additive increase in puncta formation, more than either alone

b) What is the overall conclusion from the experiment depicted in Figure 3C?

6pts
The TSP antibody depletion effectively removes TSP2 from ACM, whereas mock depletion does not (arguing that the depletion is specific for TSP).

c) What is the overall conclusion from the experiment depicted in Figure 3D?

Depletion with the TSP antibody reduces puncta forming activity, thus TSP2 is a necessary component of the activity in ACM

d) Why do the results shown in figures 4A and 4C seem somewhat surprising given the findings described earlier in the paper?

Even though the punctae contain many components of synapses (both pre- and post-synaptic), they are not fully functional (particularly post-synaptically, with regard to glutamate-induced currents).